APPENDIX 5

Scientific Abstract of the Protocol

The goal of this protocol is to assess the safety and biochemical efficacy of a recombinant adenovirus for the treatment of cystic fibrosis (CF). Although the eventual aim of such an approach is to treat the airway epithelial cells in the lungs of CF patients, the initial test will be done in the nasal epithelia. We will use nasal epithelium because it resembles that of the intrapulmonary airways, because its use allows us to apply minimal amounts of virus, and because the accessibility of the tissue allows us to measure directly the transepithelial electrical potential difference, a property that is defective in CF patients. Safety can also be assessed by removing cells and biopsy tissue for examination.

The adenovirus vector Ad2/CFTR-1 has the cDNA for CFTR inserted in place of the early region 1 genes. This renders the virus impaired for replication because the E1 genes are required for the first stages of viral infection and impaired for packaging DNA into virions because CFTR cDNA is larger than the E1 DNA it replaces. The virus can be produced in a human cell line called 293 because the cells constitutively expresses E1 proteins. Studies on the life cycle of Ad2/CFTR-1 indicate that it is severely impaired for early and late gene transcription and DNA synthesis and no Ad2/CFTR-1 virus replication has been detected in tissue culture or animals. Safety and efficacy studies in cells in culture and in animals including nonhuman primates show: 1) that Ad2/CFTR-1 can complement the chloride transport defect in human CF nasal epithelial monolayers in culture and 2) that other than a mild transient inflammatory response, Ad2/CFTR-1 has no adverse effects in hamsters and monkeys.

The protocol involves production of Ad2/CFTR-1 virus in 293 cells that have been extensively tested for adventious agents, using a viral seed stock that has been similarly tested. Following purification and further testing, the Ad2/CFTR-1 stock will be diluted to 50-200 µl and applied directly to the nasal epithelium. Virus will be applied to an area of about 0.5 cm^2 in each nostril using a small plastic applicator. After 30 minutes, the virus will be removed and the area washed. Three patients will be treated sequentially with doses of 1×10^6, 1×10^7 and 5×10^7 plaque forming units of virus.

Participants will be patients with CF who are at least 18 years old and have only mild to moderate disease. We prefer patients homozygous for the common ΔF508 mutation. We will require patients to be seropositive for adenovirus 2 antibody and to have no evidence of Ad2 or Ad5 E1 DNA sequences in their nasal epithelium. Following treatment, patients will be followed in the hospital until the virus is no longer present. Samples of nasal cells will be brushed from the treated area and tested for the presence of CFTR mRNA and protein and for the presence of replicated Ad2/CFTR-1. Measurements of the transepithelial electrical potential difference will be made of the treated and surrounding areas. Precautions to prevent spread of the virus to health care workers and the environment are described.

The successful outcome of this protocol will establish the safety and efficacy of Ad2/CFTR-1 and will be invaluable in the design of subsequent protocols and of future generations of adenovirus vector.